

# Claims:

1. A method for producing biosensors comprising at least two enzymes, for the amperometric determination of enzymatically degradable substances, such as creatinine, in biological liquids, the enzymes being immobilized on a working electrode, characterized in that an enzyme together with one or more surface-active substances in an aqueous solution is applied on the working electrode and is allowed to dry, and the at least second enzyme is chemically immobilized thereupon in a subsequent step.
2. A method according to claim 1, characterized in that polyalcohols and/or detergents, preferably non-ionic tensides, are used as surface-active substances.
3. A method according to claim 1 or 2, characterized in that the at least second enzyme is immobilized by means of crosslinking, covalent binding or matrix inclusion.
4. A method according to claim 3, characterized in that the at least second enzyme is immobilized by means of glutardialdehyde.
5. A method according to any of claims 1 to 4, characterized in that a cover membrane is applied after immobilization.
6. A biosensor comprising a working, a reference and a counter electrode, produced by means of the method according to any of claims 1 to 5, characterized in that the reference electrode is an Ag/AgCl electrode and the counter electrode is a carbon electrode and the working electrode consists of carbon, metal, metal oxides or a mixture of carbon and metal or metal oxides, the electrodes being applied on a nonconducting substrate.
7. A biosensor according to claim 6 for the determination of creatinine, characterized in that sarcosine oxidase is adsorbed on the working electrode and creatininase and creatinase are immobilized thereupon.
8. A biosensor according to claim 7, characterized in that it is made up of two three-electrodes systems, the first electrode system comprising the enzymes creatininase, creatinase and sarcosine oxidase and serving for the determination of the sum of creatinine and creatine and the second electrode system comprising the enzymes creatinase and sarcosine oxidase and serving for the determination of creatine, whereby the two results are subtracted for the determination of creatinine.
9. A biosensor according to claim 8 which comprises a further electrode system serving for the elimination of electrochemical interferences.